## **CLAIMS**

1. A conjugate comprising an erythropoietin glycoprotein having an N-terminal  $\alpha$ -amino group and one poly(ethyleneglycol), said erythropoietin glycoprotein being selected from the group consisting of human erythropoietin, analogs thereof that have from 1 to 6 additional sites for glycosylation, and human erythropoietin having at least one glycosylation site that is rearranged, and being covalently linked to one poly(ethylene glycol) group of the formula

$$-CO-(CH2)x-(OCH2CH2)m-OR,$$

wherein the -CO of the poly(ethylene glycol) group forms an amide bond with the N-terminal  $\alpha$ -amino group of the erythropoietin glycoprotein;

R is lower alkyl;

x is 2 or 3; and

m is from about 450 to about 1350.

2. The conjugate of claim 1, having the formula

$$P-NHCO-(CH2)x-(OCH2CH2)m-OR$$
 (I)

wherein P is the residue of the erythropoietin glycoprotein without the N-terminal  $\alpha$ -amino group which forms an amide linkage with the poly(ethylene glycol) group.

- 3. The conjugate of claim 1, wherein R is methyl.
- 4. The conjugate of claim 1, wherein m is from about 550 to about 1000.
- 5. The conjugate of claim 4, wherein m is from about 650 to about 750.
- 6. The conjugate of claim 4, wherein R is methyl.

## 7. The conjugate of claim 2 having the formula $CH_3O(CH_2CH_2O)_mCH_2CH_2CH_2CO\text{-NH-P}$ wherein m is from about 650 to about 750.

- 8. The conjugate of claim 1, wherein the glycoprotein is a human erythropoietin.
- 9. The conjugate of claim 8, wherein the human erythropoietin glycoprotein is expressed by endogenous gene activation.
- 10. The conjugate according to claim 8, wherein the glycoprotein has the sequence shown in Fig. 1 or Fig. 2.
- 11. The conjugate according to claim 8, wherein the glycoprotein has the sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites.
- 12. The conjugate according to claim 11, wherein the glycoprotein has the sequence of human erythropoietin which is modified by a modification selected from the group consisting of:

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Asn<sup>30</sup>Thr<sup>32</sup>;

Asn<sup>51</sup>Thr<sup>53</sup>,

Asn<sup>57</sup>Thr<sup>59</sup>;

Asn<sup>69</sup>;

Asn<sup>69</sup>Thr<sup>71</sup>;

Ser<sup>68</sup>Asn<sup>69</sup>Thr<sup>71</sup>;

Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;

Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;

Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;

Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Thr<sup>92</sup>;

Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Ala<sup>162</sup>;

Asn<sup>69</sup>Thr<sup>71</sup>Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;

Asn<sup>69</sup>Thr<sup>32</sup>Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;

Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>;
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Ser<sup>87</sup>Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>;
Asn<sup>136</sup>Thr<sup>138</sup>;
Asn<sup>138</sup>Thr<sup>140</sup>;
Thr<sup>125</sup>; and
Pro<sup>124</sup>Thr<sup>125</sup>.
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- 13. The conjugate according to claim 1, wherein the glycoprotein has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.
- 14. The conjugate of claim 13, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin and addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.
- The conjugate of claim 14, wherein the glycoprotein has the sequence of human erythropoietin modified by a modification selected from the group consisting of:

  Gln<sup>24</sup> Ser<sup>87</sup> Asn<sup>88</sup> Thr<sup>90</sup>;

  Gln<sup>38</sup> Ser<sup>87</sup> Asn<sup>88</sup> Thr<sup>90</sup>; and

  Gln<sup>83</sup> Ser<sup>87</sup> Asn<sup>88</sup> Thr<sup>90</sup>.
- 16. A pharmaceutical composition comprising the conjugate of claim 1 and a pharmaceutically acceptable excipient.
- 17. A method of treating anemia in a patient afflicted with chronic renal failure (CRF) or AIDS or resulting from chemotherapy, comprising administering to said patient an effective amount of a conjugate of claim 1.
  - 18. A process of making a conjugate of claim 1, comprising
  - a) expressing and fermenting a recombinant EPO protein that has an N-terminal peptidic extension that includes a proteolytic cleavage sequence,
  - b) protecting the  $\varepsilon$ -amino groups,

- c) proteolytically cleaving the N-terminal peptidic extension,
- d) pegylating the N-terminal α-amino group, and
- e) deprotecting the ε-amino groups of the EPO glycoprotein.
- 19. The process of claim 18 wherein the fermentation in step a) is serum free.
- 20. The process of claim 18 wherein any one of steps a)-e) is followed by a purification step.
- 21. The process of claim 18 wherein the recombinant EPO comprises a sequence selected from the group consisting of the amino acid sequences shown in any one of Figures 1, 2, 3, 4 and 5.
- 22. The process according to claim 18 wherein in step b) the  $\varepsilon$ -amino groups are protected by citraconylation.
- 23. The process of claim 18 wherein the N-terminal  $\alpha$ -amino group in step d) is pegylated with a group

$$RO(CH_2CH_2O)_m(CH_2)_xCOON$$
(II)

wherein

R is lower alkyl;

x is 2 or 3; and

m is from about 450 to about 1350.

- 24. An erythropoietin glycoprotein comprising the amino acid sequence of Fig. 1 or Fig. 2 and having an N-terminal peptidic extension that is a proteolytic cleavage site.
- 25. The erythropoietin glycoprotein of claim 24 which also comprises an N-terminal purification tag.